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# Combined effects of smoking & HPV16 in oropharyngeal cancer

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## 1    **ABSTRACT**

2    Background: Although smoking and HPV infection are recognized as important risk  
3    factors for oropharyngeal cancer, how their joint exposure impacts oropharyngeal cancer  
4    risk is unclear. Specifically, whether smoking confers any additional risk to HPV positive  
5    oropharyngeal cancer is not understood.

6    Methods: Using HPV serology as a marker of HPV-related cancer, we examined the  
7    interaction between smoking and HPV16 in 459 oropharyngeal (and 1445 oral cavity &  
8    laryngeal) cancer patients and 3024 control participants from two large European multi-  
9    center studies. Odds ratios and credible intervals [CrI], adjusted for potential  
10    confounders, were estimated using Bayesian logistic regression.

11   Results: Both smoking (OR [CrI]: 6.82 [4.52, 10.29]) and HPV seropositivity (OR [CrI]:  
12   235.69 [99.95, 555.74]) were independently associated with oropharyngeal cancer. The  
13   joint association of smoking and HPV seropositivity was consistent with that expected on  
14   the additive scale (synergy index [CrI]: 1.32 [0.51, 3.45]), suggesting they act as  
15   independent risk factors for oropharyngeal cancer.

16   Conclusions: Smoking was consistently associated with increase in oropharyngeal  
17   cancer risk in models stratified by HPV16 seropositivity. In addition, we report that the  
18   prevalence of oropharyngeal cancer increases with smoking for both HPV16-positive as  
19   well as HPV16-negative persons. The impact of smoking on HPV16-positive  
20   oropharyngeal cancer highlights the continued need for smoking cessation programs for  
21   primary prevention of head and neck cancer.

1

2   **Key message**

3   The incremental risk due to smoking in HPV16-positive oropharyngeal cancer (if any)  
4   remains unclear.

5   Pooling two large HNC studies with HPV serology data, we examined the relationship  
6   between these risk factors.

7   We demonstrate that smoking was consistently associated with increased risk of  
8   oropharyngeal cancer regardless of HPV status.

9   These data demonstrate that tobacco exposure remains an important risk factor of  
10   oropharyngeal cancer irrespective of HPV status.

11

## 1 INTRODUCTION

2 It is estimated that nearly 600,000 new cancers of the oral cavity, pharynx and larynx,  
3 are diagnosed each year worldwide, contributing to approximately 325,000 deaths each  
4 year<sup>1</sup>. Collectively referred to as head and neck cancers (HNC), most are squamous cell  
5 in origin and are grouped together due to etiologic similarities<sup>2</sup>. While nearly 33% of  
6 HNC are attributed to smoking alone, alcohol alone is estimated to cause nearly 4%,  
7 and the largest proportion of cases are attributed to the joint exposure to smoking and  
8 alcohol, nearly 35%<sup>3, 4</sup>. Recently, infection by human papillomavirus (HPV), has been  
9 associated with a subset of HNCs arising at the base of tongue, tonsils and the  
10 oropharynx<sup>5, 6</sup>.

11 HPV-positive oropharyngeal carcinoma (OPC) patients tend to be more often never  
12 smokers compared to HPV-negative patients<sup>7, 8</sup>. In addition, statistically non-significant  
13 associations between smoking and HPV-positive OPC, have led to the speculation that  
14 smoking is not an important risk factor for HPV-positive OPC<sup>9</sup>. However, it is noteworthy  
15 that up to 30% of HPV-positive HNC occurs among heavy smokers and alcohol  
16 drinkers<sup>8, 10</sup>. Further, previous analyses did not account for differing baseline risks by  
17 HPV status when interpreting the odds ratios (OR) for smoking. In addition, classification  
18 of HPV status has been an important challenge in such studies. We have previously  
19 demonstrated that circulating antibodies against HPV16 oncoprotein E6 constitute a  
20 highly specific marker of HPV16-related OPC, present in nearly 30% of OPC and less  
21 than 1% of controls<sup>11, 12</sup>, while antibodies against HPV16 capsid protein L1 are regarded  
22 as markers of past exposure<sup>13, 14</sup>. The rarity of OPC and the limited proportion of never



smokers necessitate large pooled analysis to examine the relationships between these risk factors.

A significant proportion of OPC patients report a history of smoking at diagnosis (nearly 80% in the US & EU)<sup>3, 4, 8, 15</sup>, therefore we examined whether smoking increases the risk of HPV16-positive OPC. Further, we examined the impact of smoking on the prevalence of OPC among HPV -positive and -negative persons. We also examined the association between smoking, alcohol intake, HPV16 and the risk of non-oro-pharyngeal HNC.

## **MATERIALS AND METHODS**

### Study sample

This analysis included two studies of HPV serology and HNC, the Alcohol-Related Cancers and Genetic Susceptibility in Europe (ARCAGE) study and HNC case-control study nested within the European Prospective Investigation Into Cancer and Nutrition (EPIC) cohort. Briefly, the ARCAGE study was conducted during 2002 to 2005 and included 1292 pathologically confirmed primary HNCs and 1425 controls frequency matched for age, sex, and area of residence<sup>11, 16</sup>. Ever smokers were defined as individuals who smoked any tobacco product at least once a week for a year, while ever drinkers were those who reported ever consuming any alcoholic beverage<sup>17</sup>. The EPIC cohort recruited 521,330 individuals during 1992 and 2000, of whom 385,747 participants contributed a blood sample<sup>18</sup>. This analysis included 612 incident HNC and 1599 controls<sup>12</sup>. Two controls (one in Denmark) were randomly selected for each cancer patient from appropriate risk sets consisting of all cohort participants alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case.

Controls were matched on country, sex, date of blood collection (1 month, relaxed to 5 months for sets without available controls), and date of birth (1 year, relaxed to 5 years for sets without available participants). Ever smokers were individuals who reported ever smoking any tobacco product in their lifetime, and ever drinkers were individuals who reported ever consuming any alcoholic beverage. Head neck cancers included cancers arising at the oral cavity (International Classification of Diseases for Oncology [ICD-O] C00.3–C00.9, C02.0–C06.9, C14.0–C14.9, excluding C02.4, C02.8, C02.9, C05.1, C05.2, C05.8, C05.9), oropharynx (ICD-O: C01, C02.4, C05.1–C05.2, C09, C10), hypopharynx and larynx (ICD-O: C13, C32), and non-specified and overlapping sites (ICD-O: C02.8, C02.9, C05.8, C05.9, C32.8). Lymphomas were not included, and salivary gland cancers were omitted. This analysis included head and neck cancers of all histological subtypes, of which squamous cancers comprised the vast majority (~91%), and some other rarer non-squamous histologies (6%, in ARCAGE, and 9% in EPIC). Informed consent was obtained from all participants in both the studies, and the studies were approved by the ethical review boards at the participating centers and the International Agency for Research on Cancer.

#### HPV serology

HPV antibodies were assayed using the bead-based multiplex serology method as described elsewhere<sup>19</sup>. Testing was performed blind to the case-control status of the participants. Mean fluorescence intensity (MFI) values were dichotomized by applying thresholds derived from a cross-sectional study among Korean students of mean plus 5 standard deviations (SD; for HPV16 E6) or the mean plus 3 SD excluding positive outliers (for HPV16 L1)<sup>20</sup>, as described previously<sup>11, 12</sup>.

## Statistical analysis

The overall associations between HPV16 (L1 and E6), smoking, alcohol intake and HNC risk were assessed by calculating odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). These models included age, sex, smoking status (never, former, current), alcohol consumption (never, ever plus ethanol grams/ day at recruitment), and country as covariates. Since certain combinations of exposures were very rare (e.g.: HPV16 E6-positive never smoking control subjects), Bayesian logistic regression models were used to calculate ORs and corresponding 95% credible intervals [CrI]. These models use a prior distribution to shrink or penalize the regression coefficients, thus providing more stable estimates than maximum likelihood methods. Following Gelman et al.<sup>21</sup>, all regression inputs were centered, and continuous inputs were re-scaled to have a standard deviation of 0.5. All regression coefficients were then modeled with a weakly informative Cauchy prior distribution with mean 0 and scale 2.5, with the exception of the intercept, which was given a weaker Cauchy prior with scale 10. These models were fitted using the bayesglm function in the R package ARM<sup>21, 22</sup>. In these analyses, former and current smokers were combined as ever smokers, and given the few participants who reported never consumption of any alcoholic beverage; individuals who consumed 7 grams or less of ethanol (equivalent of half a drink) per day were considered the reference. Since the results from ARCAGE and EPIC studies were similar, data were pooled in order to obtain more precise estimates. Interactions between smoking, alcohol intake and HPV16 were examined by the inclusion of an interaction term in the penalized regression models. Additive interactions were evaluated by estimating the synergy index (SI)<sup>23</sup>. The prevalence of OPC by categories of smoking

and HPV16 were calculated based on the ORs from the fitted models and assumed population prevalence of 0.003, based on the cumulative risk for pharyngeal cancer among men and women combined, in more developed regions of the world<sup>24</sup>. All statistical analyses were performed using Stata version 11.2 (Stata Corporation, College Station, TX, USA) and R version 3.1.0<sup>25</sup>.

## RESULTS

### Study profile

A total of 1904 HNC patients included 459 OPC, 1445 non-oro-pharyngeal HNCs and 3024 control subjects. Of these, 1292 HNC cases and 1425 controls were from the ARCAGE study and 612 HNC cases and 1599 controls from the EPIC study. OPC patients were similar to HNC patients as they tended to be more often men, current smokers and current drinkers (table I).

### Main effects of smoking, alcohol consumption and HPV antibodies in OPC

HPV16 E6-positivity was observed in 0.7% of 3024 controls and 31.6% of 459 OPC subjects, and was strongly associated with OPC risk (OR (95% CI): 147.31 (83.07, 361.24)). The odds of OPC was higher among current smokers compared to never smokers (OR (95% CI): 5.34 (3.89, 7.33)). Consumption of 28 or more grams of ethanol per day (2 or more drinks per day) was also associated with increased risk of OPC (OR (95% CI): 2.43 (1.77, 3.33)) (figure I).

### Combined effects of smoking and HPV16 in OPC

1 To understand the combined effects of smoking and HPV16 in OPC, we examined the  
2 relative odds of OPC given smoking and HPV16 status (table II). Compared with HPV16  
3 E6-negative never smokers, ever smoking was associated with 6.82 times increased  
4 OPC risk (95% CrI: 4.52, 10.29), while HPV16 E6-positivity alone was associated with  
5 235.69 times increased odds (95% CrI: 99.95, 555.74). HPV16 E6-positive ever  
6 smokers had 355.82 times higher OPC risk (95% CrI: 177.0, 715.30) compared to  
7 individuals negative for both risk factors. HPV16 E6-positivity and smoking appeared to  
8 interact on a less than multiplicative scale (OR [95% CrI] for the interaction term: 0.22  
9 [0.08, 0.62]). The SI for the interaction was calculated to be 1.32 (95% CrI: 0.51, 3.45),  
10 suggesting that the risks associated with HPV16 E6 and smoking might be additive.  
11 Interestingly, similar results were observed when HPV16 L1-positivity was examined  
12 (OR [95% CrI] for the multiplicative interaction term: 0.23 [0.13, 0.43], SI [95% CrI]: 0.75  
13 [0.51, 1.12]) (table II). These results remained unchanged when analyses were  
14 restricted to squamous cell cancers of the oropharynx (supplementary table V). In  
15 models stratified by HPV status (supplementary table I, model 1), we observed that OPC  
16 risk increased with the number of cigarettes smoked per day (CPD) among HPV16 L1-  
17 negatives (OR [95%CrI] for >15 CPD: 8.71 [6.01, 12.64]), as well as HPV16 E6-  
18 negatives (OR [95%CrI] for >15 CPD: 10.69 [7.06, 16.20]). Among HPV16-positives, we  
19 observed no additional increase in OPC risk with smoking dose (supplementary table I,  
20 model 1). The limited proportion of HPV16-positive controls in these comparisons is  
21 noteworthy, while 2.5% of HPV16 L1-positive controls reported smoking >15 CPD, this  
22 proportion was down to only 0.2% among HPV16 E6-positives (supplementary table I,  
23 model 2). Our previous analysis of predictors of HPV16 E6-positivity among cancer-free  
24 individuals suggested that the possibility of an underlying undiagnosed cancer cannot be

1 ruled out in such subjects<sup>26</sup>. Therefore, we excluded all HPV16 E6-positive controls  
2 under the assumption that they could be false positives or harbor other undiagnosed  
3 HPV-related cancer. We then compared HPV16 E6-positive cases with HPV16 E6-  
4 negative controls to clarify whether smoking might confer any further risk in HPV16-  
5 positive OPC (table III). Results indicate that the OPC risk among HPV16-positive  
6 persons increased with smoking. Compared with never smokers, both former smokers  
7 (OR [95% CrI]: 1.49 [0.95, 2.36]) and current smokers (OR [95% CrI]: 1.86 [1.17, 2.96])  
8 were at increased OPC risk. However, we observed a modest trend toward increased  
9 OPC risk with smoking dose (OR [95% CrI] for  $\leq 15$  CPD: 1.61 [1.03, 2.54] and OR [95%  
10 CrI] for  $>15$  CPD: 1.77 [1.11, 2.84]).

11 Relatedly, the relationship between smoking and the risk of HPV-positivity remains  
12 poorly understood. It remains unclear whether smoking may enhance HPV exposure  
13 and/or infection. Using HPV seropositivity as the end-point, Kelsey et al have recently  
14 demonstrated that younger smokers were more likely to be seropositive for HPV16 L1  
15 than older smokers<sup>27</sup>. We sought to understand whether any age-dependent association  
16 existed between smoking and HPV seropositivity in this study. Briefly, in analysis  
17 restricted to controls, age was divided into tertiles as young ( $<54$ ), intermediate (54-62)  
18 and old ( $>62$  years of age) subjects. We then examined the association between these  
19 age categories and HPV16 seropositivity by smoking status. Overall age did not appear  
20 to be strongly associated with HPV seropositivity, either HPV16 L1 (OR [95% CI]: 0.78  
21 [0.56, 1.09]) or HPV16 E6 (OR [95% CI]: 0.67 [0.22, 2.02]), albeit based on small  
22 numbers. Current smoking, on the other hand, modestly reduced the risk of HPV16 L1  
23 seropositivity (OR [95% CI]: 0.79 [0.62, 1.00]) but not HPV16 E6 (OR [95% CI]: 1.0

[0.67, 1.51]) (supplementary table VI). We found no evidence for an interaction between age and smoking towards HPV16 seropositivity (supplementary table VII). Similarly, when stratified by smoking, age was not associated with risk of HPV16 L1 seropositivity in former (OR [95% CI]: 0.91 [0.56, 1.48]) or current smokers (OR [95% CI]: 1.36 [0.79, 2.36]) (supplementary table VIII).

#### OPC prevalence by smoking and HPV16 status

In order to provide a simple appreciation of the joint importance of HPV infection and tobacco smoking, we estimated the prevalence of OPC given smoking and HPV16 E6 status, assuming an overall OPC population prevalence of 0.003. OPC prevalence among HPV16 E6-negative never smokers was negligible (0.05%; 95% CrI: 0.03%, 0.07%), which increased to 0.13% (95% CrI: 0.08%, 0.20%) in former smokers, and further increased to 0.54% (95% CrI: 0.36%, 0.80%) in current smokers. Similarly, among HPV16-positive participants, the baseline prevalence in never smokers was 8.94% (95% CrI: 4.20%, 18.01%), 11.58% (95% CrI: 5.38, 23.19%) in former smokers, and further increased to 17.68% (95% CrI: 7.29%, 36.95%) in current smokers (figure II).

#### Non-oro-pharyngeal HNC risk factors

For non-oro-pharyngeal HNC, current smoking (OR [95%CrI]: 6.82 [5.51, 8.44]), alcohol consumption of >28 grams per day (OR [95%CrI]: 2.31 [1.87, 2.85]), and HPV16 E6-positivity were associated with increased risk on non-oro-pharyngeal HNC (OR [95%CrI]: 2.57 [1.24, 5.33]), while HPV16 L1-positivity was not (OR [95%CrI]: 1.05 [0.82, 1.35]) (supplementary figure I). No interaction was observed between HPV16 and smoking for non-oro-pharyngeal HNC (supplementary table II). Alcohol consumption and HPV16

antibody status did not appear to interact to affect risk of either oropharyngeal or non-oropharyngeal HNC (supplementary tables III and IV). Study-specific associations between smoking, alcohol intake, HPV16 and OPC risk and non-oropharyngeal HNC risk are presented in supplementary figures II to V.

## **DISCUSSION**

This study supports the notion that smoking and alcohol are important risk factors for all HNC subsites, while HPV16 infection is relevant at the oropharynx. We demonstrate that smoking increases the risk of OPC, irrespective of HPV16 status. Importantly, OPC remains higher in smokers compared with never smokers, among both HPV16-positive and -negative persons.

The manner in which two (or more) distinct risk factors interact to influence disease risk can be tested by examining the joint effects. Biologically, components (or exposures) that lie within the same causal pathway are thought to interact, implying that disease will not occur (or will occur at the population baseline rates) in the absence of any one of the exposures. Statistically, the combined effect may be quantified on the multiplicative or additive scale. Most risk factors interact in greater than a multiplicative scale. For example, while smoking and alcohol consumption are each associated with substantially increased risks of HNC, smokers who also consume alcohol experience risk far greater than the product of the two<sup>3,4</sup>. Such a “supermultiplicative” risk is often interpreted to indicate that the risk factors potentiate the carcinogenic effects of each other. Truly additive effects, where the combined risk is similar to the sum of individual risks are rare. Such additive interactions are thought to indicate independence of risk factors. In this



1 study we observe that the joint effect of HPV16 (L1 and E6 antibody status) and  
2 smoking are consistent with that expected on the additive scale. This result suggests  
3 that HPV16 and smoking are independent risk factors for OPC, a conclusion supported  
4 by previous studies<sup>7, 9, 28-30</sup>. However, that the association of smoking with risk of OPC in  
5 HPV16-positives has not been statistically significant has been interpreted as the  
6 absence of risk due to smoking, and has led to the speculation that presence of HPV16  
7 could protect against the adverse effects of smoking<sup>9</sup>. In this study, we clearly  
8 demonstrate that the OPC risk increases with smoking even in the context of HPV16-  
9 positive OPC. The consistency of these observations when considering HPV16 L1 or  
10 HPV16 E6-positivity as markers of HPV16 status further strengthens our conclusions.  
11 These results would also seem to suggest that smoking may induce molecular changes  
12 in HPV16-positive OPC. This hypothesis is supported by results from the RTOG trial  
13 where OPC patients' were stratified into three distinct risk-of-death groups; while HPV-  
14 positive never smokers experienced the best prognosis and HPV-negative smokers the  
15 worst, the combined presence of smoking and HPV constituted an "intermediate" risk  
16 group<sup>31</sup>.  
17 Unlike the recently published report<sup>27</sup>, we found no evidence for an interaction between  
18 age and smoking in the risk of HPV seropositivity. This largely implies age appears not  
19 be associated with HPV seropositivity regardless of smoking status. Contrarily, the  
20 association between smoking and HPV seropositivity, either for L1 or E6, does not  
21 appear to vary with age in this study. In other words, smoking did not appear to alter  
22 either exposure or infection to HPV16 (L1 and E6, respectively) differentially by age in  
23 this study.

1 Further, our data emphasize that it remains critical to interpret relative risks in the  
2 context of the dramatically differing baseline risks between HPV16-positive and -  
3 negative people. We estimated the absolute difference in OPC prevalence between  
4 never and current smokers to be 0.5% in HPV16 E6-negative individuals, and 8.8% in  
5 HPV16 E6-positives, re-emphasizing the importance of smoking regardless of HPV16  
6 status. These data suggest that smoking remains an important risk factor for OPC, at  
7 least in Europe where almost a quarter of the patients are both HPV16 positive and  
8 report smoking history at diagnosis. In this study, we did not observe a strong interaction  
9 between alcohol consumption and HPV16. This is perhaps unsurprising given that  
10 alcohol appears to affect HNC risk at higher doses and primarily in the presence of  
11 tobacco smoking<sup>3, 4</sup>.

12 To the best of our knowledge, this constitutes the largest study examining the joint  
13 effects of smoking and HPV16 infection in OPC. It is important to note that antibody  
14 status is a systemic marker, and might therefore reflect infection in any location in the  
15 body rather than an oropharyngeal infection specifically. However, we and others have  
16 previously demonstrated HPV16 E6 as a highly specific<sup>11, 12, 28-31</sup> and likely sensitive  
17 marker for HPV16-related OPC<sup>11, 12</sup>. Further, since this uncertainty applies to both cases  
18 and controls, it is not expected to lead to biased comparisons. Further, self-reported  
19 data on alcohol and tobacco use was used, thus may contribute to some measurement  
20 error, especially in the retrospective case-control study.

21 In summary, these results indicate that smoking and HPV16 follow independent  
22 pathways towards OPC. The impact of smoking on HPV16-positive OPC may have

- 1 important implications for treatment, survival and recurrence of OPCs, and re-
- 2 emphasizes the continuing need for tobacco cessation programs.

3

4

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**Table I: Characteristics of the study population**

	ARCAGE Study			EPIC study		
	Controls	OPC	Non-OPC	Controls	OPC	Non-OPC
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
	n=1425	n=324	n=968	n=1599	n=135	n=477
<b>Age group</b>						
<55	454 (31.9)	106 (32.7)	295 (30.5)	654 (40.9)	76 (56.3)	183 (38.4)
≥55- 59	241 (16.9)	84 (25.9)	181 (18.7)	400 (25.0)	29 (21.5)	131 (27.4)
≥60- 64	210 (14.7)	53 (16.4)	155 (16.0)	325 (20.3)	24 (17.8)	113 (23.7)
≥65	520 (36.5)	81 (25.0)	337 (34.8)	220 (13.8)	6 (4.4)	50 (10.5)
<b>Sex</b>						
Men	1059 (74.3)	252 (77.8)	782 (80.8)	1105 (69.1)	89 (65.9)	351 (73.6)
Women	366 (25.7)	72 (22.2)	186 (19.2)	494 (30.9)	46 (34.1)	126 (26.4)
<b>Smoking status</b>						
Never	516 (36.2)	33 (10.2)	107 (11.1)	631 (39.5)	34 (25.2)	76 (15.9)
Former	475 (33.3)	77 (23.8)	225 (23.2)	553 (34.6)	40 (29.6)	115 (24.1)
Current	434 (30.5)	214 (66.1)	636 (65.7)	385 (24.1)	60 (44.4)	282 (59.1)
Missing				30 (1.9)	1 (0.7)	4 (0.8)
<b>Alcohol consumption</b>						
Never	172 (12.1)	11 (3.4)	62 (6.4)	55 (3.4)	3 (2.2)	14 (2.9)
Former	134 (9.4)	50 (15.4)	131 (13.5)	132 (8.3)	16 (11.9)	48 (10.1)
Current	1118 (78.5)	263 (81.2)	775 (80.1)	1025 (64.1)	93 (68.9)	319 (66.9)
Missing	1 (0.1)			387 (24.2)	23 (17.0)	96 (20.1)
<b>Level of education</b>						
Primary school	444 (31.2)	112 (34.6)	412 (42.6)	643 (41.5)	51 (38.4)	206 (44.4)
Secondary/ Technical school	834 (58.5)	193 (59.6)	518 (53.5)	604 (38.9)	56 (42.1)	190 (40.9)
University degree	147 (10.3)	19 (5.9)	37 (3.8)	302 (19.5)	26 (19.6)	68 (14.7)
Missing			1 (0.1)			
<b>Cancer site</b>						
Oral cavity	-		411 (42.5)			180 (37.7)
Oropharynx		324 (100)			135 (100)	
Larynx			534 (55.2)			247 (51.8)
Overlapping sites			23 (2.4)			50 (10.5)



**Table II: Interaction between HPV16 antibodies and smoking in the risk of oropharynx cancer**

HPV16 Antibody	Smoking status	Control N (%) (n=2994)	Oropharynx cancer N (%) (n=458)	OR [95% CrI] <sup>*</sup>
HPV16 E6 serology				
Negative	Never	1140 (38.1)	25 (5.5)	Reference
Negative	Ever	1834 (61.3)	288 (62.9)	6.82 [4.52, 10.29]
Positive	Never	7 (0.23)	42 (9.2)	235.69 [99.95, 555.74]
Positive	Ever	13 (0.43)	103 (22.5)	355.82 [177.00, 715.30]
Interaction <sup>†</sup>				0.22 [0.08- 0.62]
Synergy Index <sup>§</sup>				1.32 [0.51- 3.45]
HPV16 L1 serology				
Negative	Never	1003 (33.5)	33 (7.2)	Reference
Negative	Ever	1631 (54.5)	324 (70.7)	6.73 [4.04, 11.22]
Positive	Never	144 (4.8)	34 (7.4)	5.80 [4.03, 8.36]
Positive	Ever	216 (7.2)	67 (14.6)	9.15 [5.92, 14.13]
Interaction <sup>†</sup>				0.23 [0.13, 0.42]
Synergy Index <sup>§</sup>				0.75 [0.51, 1.12]

<sup>\*</sup> OR & credible intervals from penalized regression model on ARCAGE and EPIC studies combined

<sup>†</sup> represent OR & credible interval of multiplicative interaction evaluated by inclusion of an interaction term in the penalized regression model.

<sup>§</sup> synergy index (SI), a test of additive interaction providing evidence that the combined exposure is additive (SI=1), super additive (SI>1) or less than additive (SI<1).

**Table III: Smoking associated risk of HPV16-related oropharyngeal cancer**

	<b>Controls*</b>	<b>HPV16 E6 positive OPC</b>	
	(N=2974)	(N=145)	
<b>Exposure</b>	N (%)	N (%)	OR [95% CrI] <sup>§</sup>
<b>Smoking status</b>			
Never smoker	1140 (38.3)	42 (29.0)	Reference
Former smoker	1020 (34.3)	52 (35.9)	1.49 [0.95, 2.36]
Current smoker	814 (27.4)	51 (35.2)	1.86 [1.17, 2.96]
<b>Smoking intensity</b>			
Never smoker	1140 (41.9)	42 (30.4)	Reference
≤15 cig/day	805 (29.6)	47 (34.1)	1.61 [1.03, 2.54]
>15 cig/day	776 (28.5)	49 (35.5)	1.77 [1.11, 2.84]
<b>Alcohol consumption (grams/day)</b>			
≤7	927 (35.8)	48 (35.0)	Reference
0.7- 28	993 (38.3)	51 (37.2)	0.91 [0.59, 1.41]
>28	670 (25.9)	38 (27.7)	0.96 [0.58,1.58]

\*HPV16 E6 negative controls only were included

<sup>§</sup> adjusted for age, sex, country and alcohol consumption status or smoking status as appropriate

**Figure I: Main effects for smoking, alcohol and HPV16 antibodies and oropharyngeal cancer risk**

Legends: For HPV16, corresponding antibody negatives were considered the reference.

<sup>†</sup> 30 controls and 1 OPC patient were missing smoking status information, and 416 controls and 25 OPC patients were missing data on frequency of alcohol consumption.

\*ORs adjusted for age, sex, smoking status (never, former, current), alcohol consumption (never, ever drinkers grams per day as continuous variable) and country as appropriate.

**Figure II: Estimated prevalence of oropharyngeal cancer by smoking and HPV16 status**

Legends: The prevalence of oropharyngeal cancer by categories of smoking and HPV16 was calculated based on ORs from a model including interaction terms between smoking categories and HPV16 E6 positivity, and an assumed population prevalence of 0.003.

	Controls (N= 3024)	OPC (N= 435)	OR (95% CI)
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# HPV18 Status

L1 Seropositive	368 (12.1)	181 (22.0)	8.96 (5.37- 15.23)
L5 Seropositive	28 (0.7)	146 (31.6)	147.31(83.07-261.24)

# Smoking status<sup>a</sup>

Never smoker	1147 (38.3)	67 (14.6)	Reference
Former smoker	1028 (34.3)	117 (26.6)	2.80 (1.42- 2.82)
Current smoker	819 (27.4)	274 (59.8)	5.34 (3.89- 7.30)

# Alcohol consumption<sup>a</sup>

≤7 g/day	931 (30.7)	181 (23.3)	Reference
7- 28 g/day	1064 (35.5)	130 (30.0)	1.11(0.82- 1.50)
>28 g/day	673 (25.8)	283 (46.7)	2.43(1.77- 3.33)



